
In the Claims

Please amend the claims as follows:

1. (Original) A method for the directional subcloning of DNA fragments comprising:
 - a) providing a first vector comprising a first selectable marker gene and a DNA sequence of interest, which DNA sequence of interest is flanked by at least two restriction enzyme sites, wherein at least one of the flanking restriction enzyme sites is a site for a first restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates complementary single-strand DNA overhangs, wherein at least one of the flanking restriction enzyme sites is for a second restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates ends that are not complementary to the overhangs generated by the first restriction enzyme, wherein digestion of the first vector with the first restriction enzyme and the second restriction enzyme site generates a first linear DNA fragment which lacks the first selectable marker gene but comprises the DNA sequence of interest;
 - b) providing a second vector comprising a second selectable marker gene which is distinguishable from the first selectable marker gene and non-essential DNA sequences, optionally including a counterselectable gene, which non-essential sequences are flanked by at least two restriction enzymes sites, wherein at least one of the flanking restriction enzyme sites in the second vector is for a third restriction enzyme which generates complementary single-strand DNA overhangs that are complementary to the single-strand DNA overhang generated by the first restriction enzyme in the first linear DNA fragment, wherein at least one of the flanking restriction sites in the second vector is for a fourth restriction enzyme which generates ends that are not complementary to the ends generated by the first or third restriction enzyme but can be ligated to the ends generated by the second restriction enzyme, and wherein digestion of the second vector with the third restriction enzyme and the fourth restriction enzyme generates a second linear DNA fragment which lacks non-essential DNA sequences but comprises the second selectable marker, which second linear DNA fragment is flanked by ends which permit

the oriented joining of the first linear DNA fragment to the second linear DNA fragment; and

c) combining the first and second vectors, the first vector and the second linear DNA fragment, or the second vector and the first linear DNA fragment in a suitable buffer with one or more restriction enzymes and optionally DNA ligase under conditions effective to result in digestion and optionally ligation to yield a mixture optionally comprising a third vector comprising the first and second linear DNA molecules which are joined in an oriented manner.

2. (Original) The method of claim 1 wherein the second restriction enzyme generates blunt ends and the first linear DNA fragment is flanked by a first single-strand DNA overhang and a blunt end.

3. (Original) The method of claim 1 wherein the first and third restriction enzymes are not the same.

4. (Original) The method of claim 1 wherein the second and fourth restriction enzymes are not the same.

5. (Original) The method of claim 1 wherein the second and fourth restriction enzymes generate blunt ends.

6. (Original) The method of claim 1 wherein the first restriction enzyme is *SgfI*.

7. (Original) The method of claim 6 wherein the second restriction enzyme is *PmeI*.

8. (Original) The method of claim 1 wherein the third restriction enzyme generates a 3' TA overhang.

9. (Original) The method of claim 8 wherein the third restriction enzyme is *PvuI* or *PacI*.

-
10. (Original) The method of claim 1 wherein the DNA sequence of interest comprises an open reading frame comprising one or more sites for the first or second restriction enzyme.
11. (Original) The method of claim 10 wherein prior to digestion with the one or more restriction enzymes, the sites for the one or more restriction enzymes in the open reading frame are protected so as to prevent digestion.
12. (Original) The method of claim 11 wherein the sites are protected by methylation.
13. (Original) The method of claim 12 wherein prior to methylation the flanking sites for the first or second restriction enzyme are contacted with an oligonucleotide complementary to the flanking restriction enzyme site and RecA.
14. (Original) A vector system for cloning comprising:
a first vector comprising a first selectable marker gene and a DNA sequence of interest, which DNA sequence of interest is flanked by at least two restriction enzyme sites, wherein at least one of the flanking restriction enzyme sites is a site for a first restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates complementary single-strand DNA overhangs, wherein at least one of the flanking restriction enzyme sites is for a second restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates ends that are not complementary to the overhangs generated by the first restriction enzyme, wherein digestion of the first vector generates a first linear DNA fragment which lacks the first selectable marker gene but comprises the DNA sequence of interest, wherein the restriction enzyme sites are designed such that the first linear DNA fragment can be religated directly to a second vector comprising a second selectable marker gene which is distinguishable from the first selectable marker gene and non-essential DNA sequences, optionally including a counterselectable gene, which non-essential DNA sequences are flanked by at least two restriction enzymes sites, wherein at least one of the flanking restriction enzyme sites in the second vector is for a third restriction enzyme which

generates complementary single-strand DNA overhangs which are complementary to the single-strand DNA overhangs generated by the first restriction enzyme, wherein at least one of the flanking restriction sites in the second vector is for a fourth restriction enzyme which generates ends that are not complementary to the ends generated by the first or third restriction enzyme but can be ligated to the ends generated by the second restriction enzyme, wherein digestion of the second vector with the third and fourth restriction enzymes generates a second linear DNA fragment which lacks the non-essential DNA sequences but comprises the second selectable marker gene, wherein the second linear DNA fragment is flanked by ends which permit the oriented joining of the first linear DNA fragment to the second linear DNA fragment.

15. (Original) The vector system of claim 14 wherein the second restriction enzyme generates blunt ends and the first linear DNA fragment is flanked by a first single-strand DNA overhang and a blunt end.
16. (Original) The vector system of claim 14 wherein the first and third restriction enzymes are not the same.
17. (Original) The vector system of claim 14 wherein the second and fourth restriction enzymes are not the same.
18. (Original) The vector system of claim 14 wherein the second and fourth restriction enzymes generate blunt ends.
19. (Original) The vector system of claim 14 wherein ligation and oriented jointing yields a third vector encoding a N-terminal fusion protein which is encoded by the DNA sequence of interest and nucleic acid sequences 5' to the 3' end of the second linear DNA fragment.
20. (Original) The vector system of claim 14 wherein ligation and oriented joining yields a third vector encoding a C-terminal fusion protein which is encoded by the DNA sequence of

interest and nucleic acid sequences 3' to the 5' end of the second linear DNA fragment.

21. (Original) The vector system of claim 14 wherein ligation and oriented joining yields a third vector encoding a fusion protein which is encoded by the DNA sequence of interest and nucleic acid sequences 5' and 3' to the respective 3' and 5' end of the second linear DNA fragment.

22. (Original) The vector system of claim 14 wherein ligation and oriented joining yields a third vector encoding a fusion protein encoded by the DNA sequence of interest and the exchange site(s) created by the oriented joining.

23. (Original) The vector system of claim 14 wherein one of the restriction enzymes is *AarI*, *AscI*, *BbrCI*, *CspI*, *DraI*, *FseI*, *NotI*, *NruI*, *PacI*, *PmeI*, *PvuI*, *SapI*, *SdaI*, *SfiI*, *SgfI*, *SpII*, *SrfI*, *SwaI*, or a restriction enzyme which has the same recognition site as *AarI*, *AscI*, *BbrCI*, *CspI*, *DraI*, *FseI*, *NotI*, *NruI*, *PacI*, *PmeI*, *PvuI*, *SapI*, *SdaI*, *SfiI*, *SgfI*, *SpII*, *SrfI*, *SwaI*.

24. (Original) The vector system of claim 20, 21 or 22 wherein the fusion protein is a GST fusion protein, GFP fusion protein, thioredoxin fusion protein, maltose binding protein fusion protein, protease cleavage site fusion protein, metal binding domain fusion protein or dehalogenase fusion protein.

25. (Original) The vector system of claim 20, 21 or 22 wherein the fusion protein is more soluble, easier to purify or easier to detect relative to the corresponding non-fusion protein.

26. (Original) A kit comprising the vector system of claim 14.

27. (Original) A method for producing a vector suitable for expression of an amino acid sequence of interest, comprising:

combining at least two vectors in a suitable buffer with one or more restriction enzymes

and optionally DNA ligase under conditions effective to result in digestion and optionally ligation to yield a mixture optionally comprising a third vector, wherein a first vector comprises a first selectable marker gene and a DNA sequence of interest, which DNA sequence of interest is flanked by at least two restriction enzyme sites, wherein two or more of the flanking restriction enzyme sites are sites for a first restriction enzyme which is a hapaxotermistic restriction enzyme, wherein digestion of the first vector with the first restriction enzyme generates a first linear DNA fragment which lacks the first selectable marker gene but comprises the DNA sequence of interest and a first pair non-self complementary single-strand DNA overhangs, wherein a second vector comprises a second selectable marker gene which is distinguishable from the first selectable marker gene and non-essential DNA sequences that optionally include a counterselectable gene, which non-essential DNA sequences are flanked by two or more restriction enzyme sites, wherein two or more of the flanking sites in the second vector are for a second restriction enzyme which is a hapaxotermistic restriction enzyme, wherein digestion of the second vector with the second restriction enzyme generates a second linear DNA fragment which lacks non-essential DNA sequences but comprises the second selectable marker gene and a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs and permits the oriented joining of the first linear DNA fragment to the second linear DNA fragment.

28. (Original) A method for producing a vector suitable for expression of an amino acid sequence of interest, comprising:

combining at least two vectors in a suitable buffer with one or more restriction enzymes and optionally DNA ligase under conditions effective to result in digestion and optionally ligation to yield a mixture optionally comprising a third vector, wherein a first vector comprises a first selectable marker gene and a DNA sequence of interest, which DNA sequence of interest is flanked by at least two restriction enzyme sites, wherein at least one of the flanking restriction enzyme sites is a site for a first restriction enzyme which has infrequent restriction sites in

cDNAs or open reading frames from at least one species and generates complementary single-strand DNA overhangs, wherein at least one of the flanking restriction enzyme sites is for a second restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates ends that are not complementary to the overhangs generated by the first restriction enzyme, wherein digestion of the first vector generates a first linear DNA fragment which lacks the first selectable marker gene but comprises the DNA sequence of interest, wherein a second vector comprises a second selectable marker gene which is distinguishable from the first selectable marker gene and non-essential DNA sequences, optionally including a counterselectable gene, which non-essential DNA sequences are flanked by at least two restriction enzymes sites, wherein at least one of the flanking restriction enzyme sites in the second vector is for a third restriction enzyme which generates single-strand DNA overhangs which are complementary to the single-strand DNA overhangs generated by the first restriction enzyme, wherein at least one of the flanking restriction sites in the second vector is for a fourth restriction enzyme that which generates ends that are not complementary to the ends generated by the first or third restriction enzyme but can be ligated to the ends generated by the second restriction enzyme, and wherein digestion of the second vector with the third and fourth restriction enzymes generates a second linear DNA fragment which lacks the non-essential DNA sequences but comprises the second selectable marker gene, wherein the second linear DNA fragment is flanked by ends which permit the oriented joining of the first linear DNA fragment to the second linear DNA fragment.

29. (Original) The method of claim 28 wherein the second restriction enzyme generates blunt ends and the first linear DNA fragment is flanked by a first single-strand DNA overhang and a blunt end.

30. (Original) The method of claim 28 wherein the first and third restriction enzymes are not the same.

31. (Original) The method of claim 28 wherein the second and fourth restriction enzymes are

not the same.

32. (Original) The method of claim 28 wherein the second and fourth restriction enzymes generate blunt ends.

33. (Original) The method of claim 28 wherein one of the restriction enzymes is a class IIS restriction enzyme.

34. (Original) The method of claim 33 wherein the class IIS restriction enzyme is *AccB7I*, *AceIII*, *AclWI*, *Adel*, *AhdI*, *Alw26I*, *AlwI*, *AlwNI*, *ApaBI*, *AspEI*, *AspI*, *AsuHPI*, *BbsI*, *BbvI*, *BbvII*, *Bce83I*, *Bcefl*, *BciVI*, *BfiI*, *BglI*, *BinI*, *BmrI*, *BpiI*, *Bpml*, *BpuAI*, *BsaI*, *Bse3DI*, *Bse4I*, *BseGI*, *BseLI*, *BseRI*, *BsgI*, *BsII*, *BsmAI*, *BsmBI*, *BsmFI*, *BspMI*, *BsrDI*, *Bst71I*, *BstAPI*, *BstF5I*, *BstXI*, *Bsu6I*, *DraIII*, *DrdI*, *DseDI*, *Eam1104I*, *Eam1105I*, *EarI*, *EchHKI*, *Eco31I*, *Eco57I*, *EcoNI*, *i1396I*, *Esp3I*, *FokI*, *FauI*, *GsuI*, *HgaI*, *HphI*, *MboII*, *MsiYI*, *MwoI*, *NruGI*, *PflMI*, *PflFI*, *PleI*, *SfaNI*, *TspRI*, *Ksp632I*, *MmeI*, *RleAI*, *SapI*, *SfiI*, *TaqII*, *Tth111I*, *Tth111II*, *Van91I*, *XagI*, *XcmI*, or a restriction enzyme which has the same recognition site as *AccB7I*, *AceIII*, *AclWI*, *Adel*, *AhdI*, *Alw26I*, *AlwI*, *AlwNI*, *ApaBI*, *AspEI*, *AspI*, *AsuHPI*, *BbsI*, *BbvI*, *BbvII*, *Bce83I*, *Bcefl*, *BciVI*, *BfiI*, *BglI*, *BinI*, *BmrI*, *BpiI*, *Bpml*, *BpuAI*, *BsaI*, *Bse3DI*, *Bse4I*, *BseGI*, *BseLI*, *BseRI*, *BsgI*, *BsII*, *BsmAI*, *BsmBI*, *BsmFI*, *BspMI*, *BsrDI*, *Bst71I*, *BstAPI*, *BstF5I*, *BstXI*, *Bsu6I*, *DraIII*, *DrdI*, *DseDI*, *Eam1104I*, *Eam1105I*, *EarI*, *EchHKI*, *Eco31I*, *Eco57I*, *EcoNI*, *i1396I*, *Esp3I*, *FokI*, *FauI*, *GsuI*, *HgaI*, *HphI*, *MboII*, *MsiYI*, *MwoI*, *NruGI*, *PflMI*, *PflFI*, *PleI*, *SfaNI*, *TspRI*, *Ksp632I*, *MmeI*, *RleAI*, *SapI*, *SfiI*, *TaqII*, *Tth111I*, *Tth111II*, *Van91I*, *XagI*, *XcmI*.

35. (Original) The method of claim 33 wherein one of the restriction enzymes is *AvaI*, *Ama87I*, *BcoI*, *BsoBI*, *Eco88I*, *AvaII*, *Eco47I*, *Bme18I*, *HgiEI*, *SinI*, *BanI*, *AccB1I*, *BshNI*, *Eco64I*, *BfmI*, *BstSFI*, *SfcI*, *Bpu10I*, *BsaMI*, *BscCI*, *BsmI*, *Mva1269I*, *Bsh1285I*, *BsaOI*, *BsiEI*, *BstMCI*, *Bse1I*, *BseNI*, *BsrI*, *Cfr10I*, *BsiI*, *BssSI*, *Bst2BI*, *BsiZI*, *AspS9I*, *Cfr13I*, *Sau96I*, *Bsp1720I*, *BlpI*, *Bpu1102I*, *CelII*, *Bst4CI*, *BstDEI*, *DdeI*, *CpoI*, *CspI*, *RsrII*, *DsaI*, *BstDSI*, *Eco24I*, *BanII*, *EcoT38I*, *FriOI*, *HgiIII*, *Eco130I*, *StyI*, *BssT1I*, *EcoT14I*, *ErhI*, *EspI*, *BlpI*, *Bpu1102I*, *Bsp1720I*,

CellI, HgiAI, BsiHKAI, Alw21I, AspHI, Bbv12I, HinfI, PspPPI, PpuMI, Psp5II, SanDI, SduI, Bsp1286I, BmyI, SecI, BsaJI, BseDI, SfcI, BfmI, BstSFI, SmlI, or a restriction enzyme which has the same recognition site as *AvaI, Ama87I, BcoI, BsoBI, Eco88I, AvaII, Eco47I, Bme18I, HgiEI, SinI, BanI, AccB1I, BshNI, Eco64I, BfmI, BstSFI, SfcI, Bpu10I, BsaMI, BscCI, BsmI, Mva1269I, Bsh1285I, BsaOI, BsiEI, BstMCI, BseII, BseNI, BsrI, Cfr10I, BsiI, BssSI, Bst2BI, BsiZI, AspS9I, Cfr13I, Sau96I, Bsp1720I, BlpI, Bpu1102I, CellI, Bst4CI, BstDEI, DdeI, CpoI, CspI, RsrII, DsaI, BstDSI, Eco24I, BanII, EcoT38I, FriOI, HgiJII, Eco130I, StyI, BssT1I, EcoT14I, ErhI, EspI, BlpI, Bpu1102I, Bsp1720I, CellI, HgiAI, BsiHKAI, Alw21I, AspHI, Bbv12I, HinfI, PspPPI, PpuMI, Psp5II, SanDI, SduI, Bsp1286I, BmyI, SecI, BsaJI, BseDI, SfcI, BfmI, BstSFI, SmlI*.

36. (Original) The method of claim 1 or 28 wherein one of the restriction enzymes is *AarI, AscI, BbrCI, CspI, DraI, FseI, NotI, NruI, PacI, PmeI, PvuI, SapI, SdaI, SfiI, SgfI, SplI, SrfI, SwaI*, or a restriction enzyme that has the same recognition site as *AarI, AscI, BbrCI, CspI, DraI, FseI, NotI, NruI, PacI, PmeI, PvuI, SapI, SdaI, SfiI, SgfI, SplI, SrfI, SwaI*.

37. (Original) A method of inducing expression of a DNA sequence of interest in a host cell, comprising contacting a recombinant host cell which is deficient in rhamnose catabolism, and has a recombinant DNA molecule comprising a rhamnose-inducible promoter operably linked to an open reading frame for a heterologous RNA polymerase, with rhamnose and an expression vector comprising a promoter for the heterologous RNA polymerase operably linked to a DNA sequence of interest.

38. (Original) The method of claim 37 wherein the DNA sequence of interest is flanked by two restriction enzyme sites, wherein one of the flanking restriction enzyme sites is for a first restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates single-strand DNA overhangs, and wherein another flanking restriction enzyme site is for a second restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates ends that are not complementary to the overhangs generated by the first restriction enzyme.

-
39. (Currently Amended) A method comprising introducing a vector comprising a nucleic acid fragment encoding a barnase which lacks a secretory domain into a recombinant host cell which expresses barstar from a promoter which is constitutively ~~consitutively~~ expressed in prokaryotic cells.
40. (Original) A method comprising introducing the vector system of claim 14 into a host cell, wherein the second vector comprises a counterselectable gene comprising a nucleic acid fragment encoding a barnase which lacks a secretory domain.
41. (Original) A vector comprising an open reading frame 3' to a DNA fragment of no more than 30 base pairs, which DNA fragment comprises a ribosome binding site, a *SgfI* recognition site, and a sequence which, when present in mRNA, enhances the binding of the mRNA to the small subunit of a eukaryotic ribosome.
42. (Original) The vector of claim 41 wherein the DNA fragment includes AAGGAGCGATCGCX₁ATGX₂ (SEQ ID NO:1), and wherein X₁ and X₂ are individually an A, T, G or C.
43. (Original) A vector comprising a *SgfI* recognition site, a sequence which comprises ATG and which sequence, when present in mRNA, enhances the binding of the mRNA to the small subunit of a eukaryotic ribosome, and an open reading frame which begins at the ATG in the sequence.
44. (Original) A vector comprising a *SgfI* recognition site 5' to a recognition site for a first restriction enzyme which generates blunt ends, which vector, once digested with *SgfI* and the first restriction enzyme and ligated to a DNA fragment comprising an open reading frame flanked by an end generated by a second restriction enzyme that generates a 3' TA overhang and an end generated by a third restriction enzyme which has infrequent restriction sites in cDNAs or open

reading frames from at least one species and generates blunt ends, yields a recombinant vector comprising the open reading frame.

45. (Original) The vector of claim 44 wherein the first and third restriction enzymes are the same.

46. (Original) The vector of claim 44 wherein the first and third restriction enzymes are different.

47. (Original) The vector of claim 44 wherein the first restriction enzyme is *PmeI*, *EcoRV* or *BalI*.

48. (Original) The vector of claim 44 wherein the first restriction enzyme is *PmeI*, *DraI*, *EsaBC3I*, *HindIII*, *HpaI*, *SciI* or *SwaI*.

49. (Original) The vector of claim 44 wherein the first restriction enzyme is *AluI*, *BalI*, *BfrBI*, *BsaAI*, *BsaBI*, *BsrBI*, *BtrI*, *Cac8I*, *CdiI*, *CviJI*, *CviRI*, *Eco47III*, *Eco78I*, *EcoICRI*, *EcoRV*, *FnuDII*, *FspAI*, *HaeI*, *HaeIII*, *Hpy8I*, *LpnI*, *MlyI*, *MslI*, *MstI*, *NaeI*, *NalIV*, *NruI*, *NspBII*, *OliI*, *PmaCI*, *PmeI*, *PshAI*, *PsiI*, *PvuII*, *RsaI*, *ScaI*, *SmaI*, *SnaBI*, *SrfI*, *SspI*, *SspD5I*, *StuI*, *XcaI*, *XmnI*, or *ZraI*.

50. (Original) The vector of claim 44 wherein the restriction enzyme that generates a 3' TA overhang is *SgfI*.

51. (Original) The vector of claim 44 which further comprises an open reading frame which includes the *SgfI* site.

52. (Original) The vector of claim 44 which comprises a ribosome binding site 5' to the nucleotide cleaved by *SgfI*.

53. (Currently Amended) The vector of claim 44 wherein ligation generates the following sequence in the recombinant vector AAGGAGCGATCGCYATG (SEQ ID NO:69) or $X_1X_2X_3$ GCGATCGCCATG (SEQ ID NO:70), wherein X_1 - X_3 , X_2X_3 G or X_3 GC is a codon which is not a stop codon, and wherein Y is A, T, G or C.

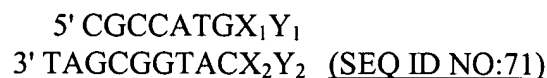
54. (Original) The vector of claim 44 wherein ligation generates the following sequence in the recombinant vector $X_1X_2X_3$ GTTTY₁Y₂, wherein $X_1X_2X_3$ is a codon in an open reading frame which is not a stop codon and Y₁ and Y₂ each =A, Y₁ = A and Y₂ = G or Y₁ = G and Y₂ = A.

55. (Original) The vector of claim 44 wherein ligation generates the following sequence in the recombinant vector $X_1X_2X_3$ GTTTY₁Y₂, wherein $X_1X_2X_3$, X_2X_3 G or X_3 GT is a codon in an open reading frame which is not a stop codon and Y₁ is not A when Y₂ is A or G, or Y₁ is not G when Y₂ is A.

56. (Original) A vector comprising a first open reading frame which includes a *SgfI* recognition site and a recognition site which is not in the open reading frame for a restriction enzyme that has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends, which vector, once digested with *SgfI* and the restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends, and ligated to a DNA fragment comprising a second open reading flanked by a single-strand 3' TA DNA overhang and a blunt end, yields a recombinant vector comprising a third open reading frame comprising the first and second open reading frames, which third open reading frame encodes a fusion peptide or protein.

57. (Currently Amended) A vector comprising a ribosome binding site which optionally overlaps by one nucleotide with a *SgfI* recognition site and a recognition site which is not in the open reading frame for a restriction enzyme that has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends, which vector, once

digested with *SgfI* and the restriction enzyme that has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends, and ligated to a DNA fragment comprising an open reading frame encoding a peptide or polypeptide flanked by



and a blunt end, yields a recombinant vector which encodes the peptide or polypeptide, wherein X_1 is the first codon which is 3' to the start codon for the open reading frame, wherein X_2 is the complement of X_1 , wherein Y_1 is the remainder of the open reading frame, and wherein Y_2 is the complement of Y_1 .

58. (Original) The vector of claim 57 wherein $X_1 = \text{GR}_1\text{R}_2$, wherein R_1 or $\text{R}_2 = \text{A, T, C or G}$.

59. (Original) A vector comprising a first open reading frame which includes a *PmeI* recognition site and is flanked at the 5' end by a recognition site for a first restriction enzyme that generates complementary single-strand DNA overhangs, which vector, once digested with *PmeI* and the first restriction enzyme, and ligated to a DNA fragment comprising a blunt end at the 5' end of a second open reading frame and an end generated by a second restriction enzyme which generates single-strand DNA overhangs which are complementary to the single-strand DNA overhangs generated by the first restriction enzyme, yields a recombinant vector comprising a third open reading frame comprising the first and second open reading frames.

60. (Currently Amended) The vector of claim 59 wherein the third open reading frame includes $\text{N}_1\text{N}_2\text{N}_3\text{GTTTN}_4\text{N}_5\text{R}$ (SEQ ID NO:72), wherein $\text{N}_1\text{N}_2\text{N}_3$ and TN_4N_5 are codons that do not code for a stop codon, and wherein R is one or more codons.

61. (Original) The vector of claim 59 wherein the blunt end of the DNA fragment is generated by a restriction enzyme other than *PmeI*.

62. (Original) The vector of claim 59 wherein the blunt end of the DNA fragment is

generated by *PmeI* digestion.

63. (Original) A vector comprising a first open reading frame which includes a *PmeI* recognition site and is flanked at the 5' end by site for a first restriction enzyme that generates complementary single-strand DNA overhangs, which vector, once digested with *PmeI* and the first restriction enzyme, and ligated to a DNA fragment comprising a blunt end and an end generated by a second restriction enzyme which generates single-strand DNA overhangs which are complementary to the single-strand DNA overhangs generated by the first restriction enzyme, yields a recombinant vector which includes $N_1N_2N_3GTTTN_4N_5$, wherein $N_1N_2N_3GTTT$ is a sequence from the 3' end of the digested expression vector, wherein $N_1N_2N_3$ do not code for a stop codon, and wherein N_4 and $N_5 = A$, or $N_4 = A$ and $N_5 = G$ or $N_4 = G$ and $N_5 = A$.

64. (Original) The vector of claim 63 wherein the blunt end of the DNA fragment is generated by *PmeI* digestion.

65. (Original) The vector of claim 63 wherein the blunt end of the DNA fragment is generated by a restriction enzyme other than *PmeI*.

66. (Original) A method for performing genetic analysis, comprising:

- a) populating a database of genetic data with a plurality of genetic records;
- b) querying the database of genetic data to identify a first subset of genetic records, wherein each record has at least one recognition site for one predetermined restriction enzyme or for restriction enzymes included in a set of predetermined restriction enzymes; and
- c) determining a set of statistics associated with the restriction enzyme recognition sites for at least a second subset of genetic records in the first subset.

67. (Original) The method of claim 66 wherein determining the set of statistics includes determining a number of genetic records including recognition sites for one predetermined restriction enzyme or for each of the predetermined restriction enzymes in the set.

-
68. (Original) The method of claim 66 wherein determining the set of statistics includes determining a number of occurrences of at least one site for the one predetermined restriction enzyme or for the predetermined restriction enzymes in a genetic record in the second subset.
69. (Original) The method of claim 66 wherein the genetic records comprise nucleic acid sequences.
70. (Original) The method of claim 66 further comprising filtering the subset of genetic records to include or exclude genetic records having one or more selected characteristics.
71. (Original) The method of claim 66 further comprising filtering the subset of genetic records to exclude genetic records having a size greater than a predetermined value.
72. (Original) The method of claim 71 wherein the predetermined value is 21000 characters.
73. (Original) The method of claim 66 further comprising determining the sequence of specific bases which are present as ambiguous bases within a recognition site or which are present between a recognition site for a restriction enzyme and the position at which the restriction enzyme cleaves DNA containing the recognition site.
74. (Original) The method of claim 66 wherein at least one of the restriction enzymes has a 6 bp, 7 bp or 8 bp recognition site.
75. (Original) The method of claim 66 wherein at least one of the restriction enzymes is a hapaxotermistic restriction enzyme.
76. (Original) A computerized system for genetic analysis, comprising:
a database of genetic data;

a processor;

a set of one or more programs executed by the processor causing the processor to;

 query the database of genetic data to identify a first subset of genetic records, wherein each record has at least one recognition site for one predetermined restriction enzyme or for restriction enzymes included in a set of predetermined restriction enzymes, and;

 determine a set of statistics associated with the restriction enzyme recognition sites for at least a second subset of genetic records in the first subset.

77. (Original) A recombinant vector prepared by digesting a vector comprising a *SgfI* recognition site 5' to a recognition site for a first restriction enzyme which generates blunt ends, with *SgfI* and the first restriction enzyme and ligating the digested vector to a DNA fragment comprising an open reading frame flanked by an end generated by a second restriction enzyme that generates a 3' TA overhang and an end generated by a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends.

78. (Original) A support comprising a plurality of recombinant vectors, one or more of which comprise a different open reading frame, wherein each recombinant vector is prepared by digesting a vector comprising a *SgfI* recognition site 5' to a recognition site for a first restriction enzyme which generates blunt ends, with *SgfI* and the first restriction enzyme and ligating the digested vector to a DNA fragment comprising an open reading frame flanked by an end generated by a second restriction enzyme that generates a 3' TA overhang and an end generated by a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends.

79. (Original) The support of claim 78 which a multi-well plate, the wells of which optionally each comprise a different recombinant vector.

PRELIMINARY AMENDMENT

Serial Number: 10/678,961

Filing Date: October 3, 2003

Title: VECTORS FOR DIRECTIONAL CLONING

Page 25

Dkt: 341.023US1

80. (Currently Amended) The support of claim 78 wherein the different open reading frames include open reading frames having nucleotide substitutions of a selected open reading frame which different open reading frames are prepared by mutagenesis ~~mutatgenesis~~ of the selected open reading frame.